HED DOC. NO. 014555

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

OXADIAZON *(THIRD REVIEW)*

FINAL REPORT

1-MAY-2001

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION: _____
Nancy McCarroll, Toxicologist

DOCUMENT PREPARATION: _____
Sanjivani Diwan, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

Karl Baetcke _____

William Burnam _____

Kerry Dearfield _____

Vicki Dellarco _____

Yiannakis Ioannou _____

Nancy McCarroll _____

Esther Rinde _____

Joycelyn Stewart _____

Clark Swentzel _____

Linda Taylor _____

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

John M. Pletcher Pathology Consultant _____

Lori Brunsman, Statistical Analysis _____

Veronique LaCapra CRM (SRRD)
Seyed Tadayon Chemist (Chem and Exposure)

CONTENTS

EXECUTIVE SUMMARY

On March 7, 2001, the Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) of the Office of Pesticide Programs met to reevaluate the carcinogenic potential of oxadiazon.

In the original carcinogenicity assessment, oxadiazon was classified as a **Group B2**, probable human carcinogen, based on the increased incidence of malignant or combined benign and malignant liver tumors in multiple species (CD -1 mice and F344 rats of one or both sexes) and in multiple experiments (two mouse studies and one rat study (1984; HED Document No. 004097). On September 3, 1986 during the 2nd meeting, the Toxicology Branch Peer Review Committee reaffirmed the earlier classification of oxadiazon as a Group B2 carcinogen but there was a minority opinion that the agent should be placed in Group C, possible human carcinogen (1987; HED Document No. 007798). Based on weight-of-the-evidence for oxadiazon, the Scientific Advisory Panel reiterated the minority view (1987; HED Document No. 007798). Subsequently, the cancer classification for oxadiazon was revised to a **Group C carcinogen** and for the quantification of human cancer risk, a linear low dose extrapolation approach was recommended (1987; HED Document No. 007798). The decision to reclassify oxadiazon as a Group C carcinogen was based on the rationale that liver tumors were produced in two of the three positive studies (one mouse study and one rat study) at doses that exceeded the maximum tolerated dose (MTD).

At the March 7, 2001 CARC meeting, information/data previously not available to the Peer Review Committee were considered which included chronic toxicity/carcinogenicity studies in Wistar rats and ICR-JCL mice. In the rat study, oxadiazon was administered in the diet to groups of 80 male and 80 female Wistar rats at concentrations of 0, 3, 10, 100 or 1000 ppm (0, 0.106, 0.36, 3.5 or 39 mg/kg/day for males and 0, 0.131, 0.44, 4.2 or 44 mg/kg/day for females, respectively) for up to 104 weeks. In the mouse study, oxadiazon was administered to groups of 80 male and 80 female ICR-JCL mice at dietary concentrations of 0, 3, 10, 100 or 1000 ppm (0, 0.315, 1.09, 10.6 or 113 mg/kg/day for males and 0, 0.278, 0.92, 9.3 or 99 mg/kg/day for females, respectively) for 98/99 weeks. The Registrant also submitted mechanistic studies to support the proposed mode of action for liver tumor induction observed in these studies.

The CARC concluded that:

- **There was <u>clear evidence</u> that oxadiazon was carcinogenic to male Wistar rats** because: 1) There were statistically significant positive trends for liver adenomas, carcinomas and combined adenomas/carcinomas. There was a statistically significant increase by pair-wise comparison with the controls for liver adenomas and combined liver adenomas/carcinomas at 100 and 1000 ppm and for liver carcinomas at 1000 ppm indicating a malignant component to the liver tumors; and 2) The incidences of liver adenomas at 100 ppm and 1000 ppm and carcinomas at 1000 ppm were outside the published range of spontaneous incidences in Wistar rats (range: adenomas, 0%-2.5%; carcinomas, 0%-2.5%). The highest dose level tested in this study was considered to be

adequate and not excessive in male rats because there were decreases in body weight gains and the clinical and histopathological liver changes observed were not severely adverse. The survival of the animals was not affected by the treatment. There was a statistically significant increasing trend for liver carcinomas in females but there was no significant increase in liver tumors in treated females by pair-wise comparisons with controls. The Committee determined that, for female rats, the highest dose was adequate based on an increased incidence of chronic nephropathy. **The CARC concluded that the increased incidence of liver tumors observed in the male rats was treatment-related.**

- **There was clear evidence that oxadiazon was carcinogenic to male and female ICR-JCL mice** because: There were statistically significant positive trends for liver adenomas, carcinomas and combined adenomas/carcinomas in both sexes. There were also statistically significant increases by pair-wise comparison of the dosed groups with the controls for liver adenomas and carcinomas as well as for combined liver adenomas/carcinomas at 100 and 1000 ppm for males and at 1000 ppm for females. There was a malignant component to the liver tumors in both sexes. The highest dose level tested was considered to be adequate and not excessive in both sexes based on increased liver weights and histopathological changes in the liver at 1000 ppm which were not severely adverse. **The Committee concluded that there were treatment-related increases in both benign and malignant liver tumors in male and female mice.**

  The positive results of an *in vitro* cell transformation assay are in concordance with the findings of *in vivo* rodent bioassays.

- A battery of acceptable mutagenicity assays indicated that oxadiazon was not mutagenic.

- The mechanistic studies provide insufficient data to determine whether a threshold mechanism exists for the induction of liver tumors observed in rats and mice. Nevertheless, the formation of brown pigment in the liver and kidneys of rats and mice is consistent with the known inhibitory action of oxadiazon toward protoporphyrinogen oxidase, a critical enzyme in chlorophyll and heme biosynthesis.

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified oxadiazon into the category **"Likely to be carcinogenic to humans"** based on the following weight-of-the-evidence considerations:

1. Treatment-related benign and malignant liver tumors were observed in two species. There was clear evidence that oxadiazon induced a statistically significant increase in liver tumors in male Wistar rats and male and female ICR-JCL mice. The findings of liver tumors are consistent with the results of earlier studies in male F-344 rats and male and female CD mice. The positive results from an *in vitro* cell transformation assay are in concordance with the results of *in vivo* rodent bioassays.

2. Oxadiazon was not mutagenic. However, it causes cell transformation *in vitro;* these results are in concordance with the carcinogenicity seen in *in vivo* rodent studies.

The Committee recommended a low dose linear extrapolation approach for the quantification of human cancer risk based on the most potent liver tumors in rats and mice. This approach is supported by the inadequacy of data on the mode of action for oxadiazon-induced liver tumors in rodents.

I. INTRODUCTION

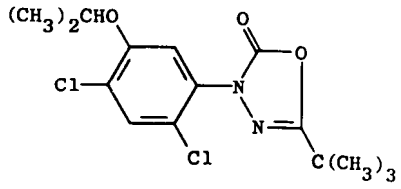
According to the original carcinogenicity assessment for oxadiazon, the pesticide was ranked as a **Group B2**, probable human carcinogen (1984; HED Document No. 004097). The rationale for the classification, Group B2, was based on the increased incidence of malignant or combined malignant and benign liver tumors in multiple species (CD -1 mice and F344 rats of one or both sexes) and in multiple experiments (two mouse studies and one rat study). The Toxicology Branch Peer Review of this agent was later held on September 3, 1986 and the Committee affirmed the earlier classification of oxadiazon as a Group B2 carcinogen but there was a minority opinion that the agent should be placed in Group C, possible human carcinogen (1987; HED Document No. 007798). Review of the weight-of-the-evidence on oxadiazon by the Scientific Advisory Panel reiterated this minority view (1987; HED Document No. 007798). At that time, the Agency's decision on the carcinogenic potential of oxadiazon concurred with the Scientific Advisory Panel's (SAP) classification of oxadiazon as a **Group C carcinogen** (1987; HED Document No. 007798)**.** The decision to reclassify oxadiazon as a Group C carcinogen was based on the rationale that liver tumors were produced in two of the three positive studies (one mouse study and one rat study) at doses that exceeded the maximum tolerated dose (MTD). For the quantification of human cancer risk a linear low dose extrapolation approach was recommended ($Q1^* = 1.4 \times 10^{-1} (mg/kg/day)^{-1}$).

On March 7, 2001, the Cancer Assessment Review Committee (CARC) met to reconsider the carcinogenicity classification of oxadiazon under the draft Agency Cancer Risk Assessment Guidelines (1999) for the human cancer risk assessment. At this meeting, information/data previously not available or relevant to this review were presented by Nancy McCarroll of the Toxicology Branch. These include a chronic/carcinogenicity toxicity study in Wistar rats (MRID No. 40993401), a carcinogenicity study in ICR-JCL mice (MRID No. 40993301), genetic toxicology as well as mechanistic studies and data on structurally-related compounds. Based on the available studies, the quantitative risk to humans was also evaluated.

II. BACKGROUND INFORMATION

Oxadiazon (P.C. Code: 109001, CAS Number: 19666-30-9, 5-tert-butyl-4-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol-2-one), also known as *Ronstar*, is a selective pre-emergent and early post emergence herbicide that is effective primarily for the control of annual grasses and broadleaf weeds in turf. It has no food or feed uses. Aventis CropScience USA is supporting use of oxadiazon on golf courses, ornamentals, apartment/condo lawns, athletic fields, parks, playgrounds and cemeteries. Oxadiazon destroys cell membranes and inhibits photosynthesis, probably by generating oxidizing radicals in the presence of light and is a powerful inhibitor of plant, yeast and mouse protoporphyrinogen oxidase, an enzyme critical in the biosynthesis of chlorophyll and heme (Matringe *et al.*, 1989).

. Figure 1. Chemical Structure of Oxadiazon



III. EVALUATION OF CARCINOGENICITY AND OTHER EVIDENCE

4. Previous Chronic Toxicity/Carcinogenicity Studies

Earlier tumor data for Oxadiazon in F-344 rats and CD-1 mice were discussed previously (HED Document NO. 007798, dated August 27, 1987) and are not reiterated here.

2. Combined Chronic Toxicity/ Carcinogenicity Study in Wistar Rats

Reference

Y. Shirasu (1987). Oxadiazon - 24 Month Chronic Toxicity and Carcinogenicity Study in Rats. Institute of Environmental Toxicology, Tokyo, Japan; Study No. Not listed; Report dated February 1987. (Unpublished) MRID: 40993401

A. Experimental Design

Oxadiazon (95.9%) was administered to SPF Wistar rats (80/sex/dose) in the diet at dose levels of 0, 3, 10, 100 or 1000 ppm (equivalent to 0, 0.106, 0.36, 3.5 or 39 mg/kg/day for males or 0, 0.131, 0.44, 4.2 or 44 mg/kg/day for females) for 104 weeks. All 80 rats/sex/dose were reportedly examined for histopathology.

B. Discussion of Tumor Data

The incidences of liver tumors in male and female rats are presented in Tables 1 and 2, respectively. For males, there were statistically significant ($p<0.01$) increasing trends for liver adenomas, carcinomas and combined adenomas/ carcinomas (Table 1). There were also significant increases in the pair-wise comparisons with the controls for liver adenomas at 100 ppm ($p<0.05$) and 1000 ppm ($p<0.01$), carcinomas at 1000 ppm ($p<0.05$) and combined adenomas/carcinomas at 100 ppm ($p<0.05$) and 1000 ppm ($p<0.01$). For female rats, there was a statistically significant increasing trend ($p<0.05$) for liver carcinomas only and no significant increases in pair-wise comparisons of the treated groups with the controls were noted for liver adenomas, carcinomas and combined adenomas/carcinomas (Table 2).

Historical control data were not provided by the performing laboratory; however, the spontaneous incidences of liver tumors in Wistar rats reported by Welsh and Poteracki (1994) are as follows: males-- adenomas: 1.02%, range 0-2.5%; carcinomas: 0.88%, range 0-2.5%; females- adenomas: 2.34%, range 0-12%; carcinomas: 0.88%, range 0-10%. The incidences of adenomas at 100 and 1000 ppm (9% and 13% , respectively) and carcinomas (10%) at 1000 ppm in males exceeded the range of available spontaneous incidences. The incidences of liver carcinomas (4%) in females did not exceed the range of available spontaneous incidences. **The Committee concluded that the increase in liver tumors in male rats was treatment related.**

C. Non-neoplastic Lesions

In 1991, the Agency requested the Registrant to provide new pathology summary tables and indicate the exact number of each tissue that was examined (HED Document No. 008949) because the exact number of tissues/organs examined could not be verified by the Reviewers. No correspondence regarding this request has been presented to HED. However, the recent review of data by the HED statistician indicated that none of the animals were excluded from the analyses. Therefore, the Committee concluded that the integrity of the study or the interpretation of the results is not compromised by this data deficiency.

The histopatholgical examination revealed increases in non-neoplastic changes in the liver at 1000 ppm. These included: centrilobular liver swelling (♂ and ♀); acidophilic foci of cellular alteration (♂); and brown pigmentation in the Stellate cells (♂ and ♀) (Table 3). At 100 ppm, a significant ($p<0.05$) increase in the number of males with centrilobular liver swelling was seen. Brown pigmentation in the proximal tubular cells (♂ and ♀) and in cortical interstitial tissue (♂) as well as chronic nephropathy (♀) were also noted in the kidneys of high-dose rats.

Table 1. Oxadiazon - SPF Wistar Rat Study

Male Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)- (Brunsman, 2001)

	Dose (ppm)				
	0	3	10	100	1000
Adenomas (%)	0/53 (0)	1/55 (2)	1/54 (2)	5[a]/54 (9)	7[a] /52 (13)
p =	0.001**	0.509	0.505	0.030*	0.006**
Carcinomas (%)	0/53 (0)	0/55 (0)	0/54 (0)	0/54 (0)	5[b]/52 (10)
p=	0.000**	1.000	1.000	1.000	0.027*
Combined (%)	0/53 (0)	1/55 (2)	1/54 (2)	5/54 (9)	12/52 (23)
p =	0.000**	0.509	0.505	0.030*	0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

[a] First adenoma observed simultaneously at week 78 in interim sacrifice animals, doses 100 and 1000 ppm.

[b] First carcinoma observed at week 78 in an interim sacrifice animal, dose 1000 ppm.

Note: 26- and 56-week interim sacrifice animals are not included in this analysis. There were no liver tumors in any interim sacrifice animals at 26 or 52 weeks.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$.

If **, then $p < 0.01$.

Table 2. Oxadiazon - SPF Wistar Rat Study:

Female Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)- (Brunsman, 2001)

	Dose (ppm)				
	0	3	10	100	1000
Adenomas (%)	1[a]/52 (2)	1/52 (2)	1/55 (2)	1/53 (2)	1/55 (2)
p =	0.550	0.752	0.738	0.748	0.738
Carcinomas (%)	0/52 (0)	0/52 (0)	0/55 (0)	0/53 (0)	2[b]/55 (4)
p=	0.042*	1.000	1.000	1.000	0.262
Combined (%)	1/52 (2)	1/52 (2)	1/55 (2)	1/53 (2)	3/55 (5)
p =	0.098	0.752	0.738	0.748	0.330

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.
[a] First adenoma observed at week 103, dose 0 ppm.
[b] First carcinoma observed at week 104, dose 1000 ppm

Note: 26 - and 52-week interim sacrifice animals are not included in this analysis. There were no liver tumors in any in terim sacrifice animals at 26 and 56 weeks.
Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$.

If **, then $p < 0.01$.

Table 3. Non-neoplastic Lesions in the Liver of Wistar Rats Fed Dietary Administrations of Oxadiazon for 104 Weeks

Lesions of the Liver	Males[a] Group (ppm)					Females[a] Group (ppm)				
	0	3	10	100	1000	0	3	10	100	1000
Centrilobular hepatocellular Swelling	1	2	1	8*	52*	3	2	1	9	33**
Foci of Cellular Alteration (acidophilic)	9	14	14	9	18*	12	10	8	14	9
Brown Pigmentation of Stellate Cells	0	0	0	1	35***	4	1	4	1	12**
Focal Hepatocellular Fatty Change	3	7	7	10*	8	9	10	3	8	5

[a] 80 animals/sex were reportedly examined at each dose level.
* Significantly different ($p<0.05$) than the vehicle control by Fisher's Exact Test.
** Significantly different ($p<0.01$) than the vehicle control by Fisher's Exact Test.
***Significantly different ($p<0.001$) than the vehicle control by Fisher's Exact Test.

Selected non-neoplastic changes in the kidney, which were considered to be treatment-related, are shown in Table 4. At 1000 ppm, these included: brown pigmentation in the proximal tubular cells ($p<0.001$, ♂♀), brown pigmentation in the cortical interstitial tissue ($p<0.001$, ♂), and chronic nephropathy ($p<0.01$, ♀). At lower doses, non-neoplastic lesions were comparable to the vehicle control values.

Table 4. Non-neoplastic Lesions in the Kidneys of Wistar Rats Fed Dietary Administrations of Oxadiazon for 104 Weeks

Lesions of the Kidneys	Males[a] Group (ppm)					Females[a] Group (ppm)				
	0	3	10	100	1000	0	3	10	100	1000
Brown Pigmentation in Proximal Tubular Cells	3	4	1	2	50***	6	5	8	13	20***
Brown Pigmentation in Cortical Interstitial Tissue	2	0	0	1	49***	0	0	1	1	2
Chronic Nephropathy	28	28	33	36	37	11	16	15	14	26**

[a] 80 animals/sex were reportedly examined at each dose level.
* Significantly different ($p<0.05$) than the vehicle control by Fisher's Exact Test.
** Significantly different ($p<0.01$) than the vehicle control by Fisher's Exact Test.
***Significantly different ($p<0.001$) than the vehicle control by Fisher's Exact Test

C. Adequacy of Dosing for Assessment of Carcinogenic Potential

Statistical evaluation of mortality indicated no significant incremental changes with increasing doses of oxadiazon in the male and female rats (Brunsman, 2001). **The dosing at the highest dose (1000 ppm) was considered to be adequate and not excessive in male rats** based on decreased body weight gain (generally throughout the study), signs of transient anemia (evident at week 26), increased serum enzyme activity (LDH ↑≥63%; ALP ↑245%; SGOT ↑ 151%; SGPT ↑ 646%), increased bilirubin and increased liver and kidney weights with associated pathological changes at 1000 ppm which were not severely adverse (Refer to Table 3 for details).

For females at 1000 ppm, there was a decrease in the terminal body weight (-8.9%). However, the findings were confounded by apparently fairly large standard deviations. With the exception of decreased terminal body weight, there were no significant differences from controls in body weight measurements. Significant increases in liver and kidney weights were seen only at week 26 and 78 (kidney only). While increases in liver weight and findings of liver pathology were reported for females at 1000 ppm, these effects were either less consistent or occurred less frequently than those observed in males at this level. Other findings (anemia, increased serum enzymes and pathological change such as foci of cellular alteration) showing evidence of toxicity, which were generally confined to the high dose were not noted in females. However, there was a significant increase in the incidence of chronic nephropathy among high dose females. **The Committee, therefore, concluded that the highest dose tested in female rats was adequate to assess the carcinogenic potential of oxadiazon.**

2. Combined Chronic Toxicity/Carcinogenicity Study in ICR-JCL Mice

Reference

Y. Shirasu (1987). Oxadiazon-23 Month Chronic Toxicity and Oncogenicity Study in Mice. Institute of Environmental Toxicology, Tokyo, Japan; Study No. Not listed; Report dated February 1987. (Unpublished) MRID: 40993301

A. Experimental Design

Oxadiazon (95.9%) was administered to 80 ICR-JCL mice (80/sex/dose) in the diet at 0, 3, 10, 100 or 1000 ppm (equivalent to 0, 0.315, 1.09, 10.6 or 113 mg/kg/day for males or 0, 0.278, 0.92, 9.3 or 99 mg/kg/day for females) for 98-99 weeks (the study was scheduled to run for 104 weeks but due to deaths, it was terminated at 98-99 weeks). Groups of 9-10 mice/sex/group were sacrificed at weeks 52 were subjected to pathology analysis.

B. Discussion of Tumor Data

The incidences of liver tumors in male and female mice are presented in Table 5 and 6, respectively. For male mice, statistically significant increasing trends ($p<0.01$) were observed for liver adenomas, carcinomas and combined adenomas/carcinomas (Table 5). There were also significant increases by pair-wise comparisons of the 100 and 1000 ppm dose groups with the controls for liver adenomas, combined adenomas/carcinomas (both at $p <0.01$) and carcinomas ($p <0.05$ and $p<0.01$, respectively). No significant increases were noted at lower doses (3 or 10 ppm). For female mice, statistically significant increasing trends ($p<0.01$) were noted for liver adenomas, carcinomas and combined adenomas/carcinomas (Table 6). There were statistically significant increases by pair-wise comparison of the 1000 ppm dose group with the controls for adenomas ($p<0.01$), carcinomas ($p <0.05$) and combined adenomas/carcinomas ($p <0.01$). Historical control data were not provided by the performing laboratory.

Table 5. Oxadiazon - ICR-JCL Mouse Study:

Male Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)- (Brunsman, 2001)

	Dose (ppm)				
	0	3	10	100	1000
Adenomas (%)	2/69 (3)	7/71 (10)	2/71 (3)	12/69 (17)	16[a]/71 (23)
p =	0.000**	0.090	0.676	0.005**	0.000**
Carcinomas (%)	3/69 (4)	1/71 (1)	4/71 (6)	11/69 (16)	29[b]/71 (41)
p=	0.000**	0.299	0.516	0.023*	0.000**
Combined (%)	5/69 (7)	8/71 (11)	6/71 (8)	23/69 (33)	45/71 (63)
p =	0.000**	0.300	0.520	0.000**	0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 46.

[a] First adenoma observed at week 52 in an interim sacrifice animal, dose 1000 ppm.

[b] First carcinoma observed at week 46, dose 1000 ppm.

Note:
Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 6. Oxadiazon - ICR-JCL Mouse Study:
Female Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results
(p values)- (Brunsman, 2001)

	Dose (ppm)				
	0	3	10	100	1000
Adenomas	0/52	0/53	1[a]/46	1[a]/48	8[a]/51
(%)	(0)	(0)	(2)	(2)	(16)
p =	0.000**	1.000	0.469	0.480	0.003**
Carcinomas	1/52	0/53	0/46	1/48	7[b]/51
(%)	(2)	(0)	(0)	(2)	(14)
p=	0.000**	0.495	0.531	0.732	0.028*
Combined	1/53	0/53	1/46	2/48	15/51
(%)	(2)	(0)	(2)	(4)	(29)
p =	0.000**	0.495	0.721	0.470	0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

[a] First adenoma observed at week 99 in final sacrifice animal simultaneously in doses of 10, 100 and 1000 ppm.

[b] First carcinoma observed at week 83, dose 1000 ppm.

Note: Interim sacrifice animals are not included in this analysis. There were no liver tumors in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

In addition to liver tumors in females, a significant ($p<0.05$) increase by pair-wise comparison of the 1000 ppm dose group with the controls was noted for malignant lymphoma (Table 7); however, no dose-response was evident. **The Committee concluded that these tumors were not treatment-related because there was no dose-response, the tumor response was variable and these tumors were not seen in the male mice. In addition, these tumors were also not reported in the previously performed mouse carcinogenicity and rat chronic/ carcinogenicity studies with oxadiazon.** The historical control data were not available for comparison.

Table 7. Oxadiazon - ICR-JCL Mouse Study:
Female Lymphoma Tumor Rates[+] and Exact Trend Test and
Fisher's Exact Test Results (p values)- (Brunsman, 2001)

	Dose (ppm)				
	0	3	10	100	1000
Malignant Lymphoma (%)	16/80 (20)	25/79 (32)	19/80 (24)	21[a]/80 (26)	27/80 (34)
p =	0.067	0.067	0.351	0.227	0.037*

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 19.
[a] First malignant lymphoma observed at week 19, dose 100 ppm.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-neoplastic Lesions:

In 1991, the Agency requested the Registrant to provide new pathology summary tables and indicate the exact number of each tissue that was examined (HED Document No. 008949) because the exact number of tissues/organs examined could not be verified by the Reviewers. No correspondence regarding this request has been presented to HED. However, the recent review of data by the HED statistician indicated that none of the animals were excluded from the analyses. Therefore, the Committee concluded that the integrity of the study or the interpretation of the results is not compromised by this data deficiency.

The non-neoplastic changes in the liver observed in male and female mice are listed in Table 8. At 1000 ppm, there was a significant increase in the incidence of centrilobular liver swelling ($p<0.001$ ♀), diffuse liver swelling ($p<0.001$♂ and ♀), brown pigment disposition ($p<0.001$♂ and ♀), and bile duct proliferation ($p<0.001$♂). The incidence of diffuse liver swelling and brown pigment disposition was also significantly increased at 100 ppm ($p<0.001$) but only in the males. The formation of brown pigment in the liver and kidneys of male mice is consistent with the inhibition of porphyrin biosynthesis by oxadiazon.

Other treatment-related findings included a significant increase in the incidence of auricular hardening or thrombus in the heart of high-dose males ($p<0.05$) and a significant ($p<0.001$) increase in brown pigment disposition in the proximal tubules of high-dose male kidneys.

Table 8. Non-neoplastic Lesions in the Liver of ICR-JCL Mice Fed Dietary Administrations of Oxadiazon for 98-98 Weeks

Lesions of the Liver	Males[a] Group (ppm)					Females[a] Group (ppm)				
	0	3	10	100	1000	0	3	10	100	1000
Centrilobular Hepatocellular Swelling	1	1	0	3	2	1	0	1	1	14***
Diffuse Hepatocellular Swelling	1	2	5	45***	67***	3	5	1	2	24***
Brown Pigmentation Disposition	4	3	5	47***	59***	0	1	0	3	35***
Extra medullary Hematopoiesis	1	2	6	1	0	13	10	7	6	4*
Diffuse Hepatocellular Necrosis	1	2	4	16***	7*	0	0	0	0	0
Bile Duct Proliferation	0	0	0	1	15***	0	0	0	0	0

[a] 80 animals/sex were reportedly examined at each dose level.
* Significantly different ($p<0.05$) than the vehicle control by Fisher's Exact Test.
** Significantly different ($p<0.01$) than the vehicle control by Fisher's Exact Test.
***Significantly different ($p<0.001$) than the vehicle control by Fisher's Exact Test.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

Statistical evaluation of mortality indicated no significant incremental changes with increasing doses of oxadiazon in the male and female rats (Brunsman, 2001). The CARC considered the dosing to be adequate and not excessive in males and females based on anemia and pathological changes in the liver at the highest dose tested (HDT) which were not severely adverse (Refer to Table 8 for details). The treatment related effects observed at 1000 ppm (HDT) included transient anemia (evident at week 52) in males and females, increased serum enzymes (SGPT (↑275-300%) , SGOT (↑117%), ALP (↑325%) and BUN (↑44%) (♂ and ♀) and at 100 ppm: increased SGPT (↑147%) (♂)), increased liver weights (absolute and relative) in males at week 52 and 98 and in females at week 98 and increased absolute and relative adrenal (♂ -- week 98) and kidney (♀ -- week 98) weights.

IV. OTHER TOXICOLOGY DATA

A. Mutagenicity and Cell Transformation

Overall, the data indicate that oxadiazon is not mutagenic but does cause neoplastic cell transformation *in vitro* **in Syrian hamster kidney BHK21 C13/HRC1 cells.** Nine acceptable mutagenicity studies were available for review. These studies satisfy the pre-1991 FIFRA guideline requirements . The summaries of these studies are presented below:

GENE MUTATION

a) *Salmonella typhimurium/Escherichia coli* reverse gene mutation assay. The assay was **negative** in *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 and *E. coli* WP2 *hcr*⁻ up to the highest dose tested (2500 μg/plate -S9; 1000 μg/plate +S9) of 99.18% oxadiazon (MRID No. 00069893).

b) *S. typhimurium* reverse gene mutation assay: The assay was **negative** in *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 exposed to 97.49% oxadiazon up to 5000 μg/plate+/-S9; cytotoxicity was seen at ≥3330 μg/plate -S9 (MRID No. 41871701).

c) L5178Y TK +/- mouse lymphoma cell/mammalian activation forward mutation assay: The assay was **negative** in cells treated with oxadiazon (95.5% a.i.) up to reproducibly cytotoxic levels in the absence of S9 activation (1000 μg/mL) and severely cytotoxic doses (≥200 μg/mL) with S9 activation. Oxadiazon was insoluble at ≥62.5 μg/mL (MRID No. 00115726).

d) L5178Y TK +/- mouse lymphoma cell/mammalian activation forward mutation assay: The assay was **negative** in cells treated with recrystallized oxadiazon (100% a.i.) up to cytotoxic levels (1000 μg/mL -S9; 250 μg/mL +S9; MRID 00115729).

CHROMOSOME ABERRATIONS

e) *In vitro* chromosome aberration assay in Chinese hamster ovary (CHO) cells: The assay was **negative** in cells treated with oxadiazon (95.5% a.i.) up to cytotoxic concentrations (75 μg/mL -S9; 41.6 μg/mL +S9) and the limit of solubility ($\geq$416 μg/mL) (MRID 00115730).

f) *In vitro* chromosome aberration assay in Chinese hamster ovary (CHO) cells: The assay was **negative** in cells treated with recrystallized oxadiazon (100% a.i.) up to cytotoxic concentrations (200 μg/mL -S9; 500 μg/mL +S9) and the limit of solubility (667 μg/mL -S9; 200 μg/mL +S9; MRID 00115728).

OTHER MUTAGENIC MECHANISMS

g) Unscheduled DNA Synthesis(UDS) in primary rat hepatocytes assay: The test was **negative** in hepatocytes exposed to oxadiazon (95.5% a.i.) to cytotoxic concentrations ($\geq$100 µg/mL) and the limit of solubility ($\geq$50 µg/mL) (MRID No. 00115727).

h) UDS in primary rat hepatocytes assay: The test was **negative** in hepatocytes exposed to recrystallized oxadiazon (100% a.i.) up to cytotoxic concentrations (100-500 µg/mL) and the limit of solubility ($\geq$25 µg/mL) (MRID No. 00115723).

CELL TRANSFORMATION

i) *In vitro* cell transformation assay in Syrian hamster kidney BHK21 C13/HRC1 cells: The test was **positive both with and without S9 activation**, based on the induction of transformation frequencies (TFs) $\geq$5 times the solvent control value at the LD_{50} . Oxadiazon (90% a.i.) and recrystallized oxadiazon (100 % a.i.) were tested up to cytotoxic concentrations with LD_{50} values in the absence of S9-mix of 118 µg/mL and 200 µg/mL, respectively. In the presence of S9-mix, the LD_{50} of oxadiazon was 69 µg/mL; however, the LD_{50} for recrystallized oxadiazon was not determined as cell viability was 78% of the solvent control at the highest dose tested (400 µg/mL). The transformation frequencies (the number of transformed colonies/10^6 surviving cells) at the LD_{50} concentrations were 128 and 79 for cells treated with oxadiazon in the absence and presence of S9-mix, compared to the solvent control values of 4 and 5, respectively. Recrystallized oxadiazon induced transformation frequencies of 55 at the LD_{50} in the absence of S9-mix and 60 at the highest dose tested in the presence of S9-mix. A positive dose-response trend was generally apparent for both concentrations. This study is classified as acceptable (nonguideline) (MRID No. 00115703).

2. Conclusions

Acceptable bacterial assays with ≥97.49% oxadiazon were negative for gene mutations in *Salmonella typhimurium* and *Escherichia coli* (MRID Nos. 00069893 and 41871701). Similarly, neither 95.5% oxadiazon nor recrystallized Oxadiazon (100%) were mutagenic or clastogenic in cultured mammalian cells (MRID Nos. 00115726, 00115729, 00115728 and 00115730) and did not cause UDS in primary rat hepatocytes (MRID Nos. 00115727 and 00115723). There is, however, evidence that both formulations induced neoplastic transformation in Syrian hamster kidney cells both in the presence and in the absence of S9 activation (MRID No. 00115703). The finding of positive cell transformation supports the evidence from mouse bioassays (MRID Nos. 00444322, 00115733 and 40993301) and the rat long-term studies (MRID Nos. 00149003/00157780 and 40993401) of liver tumor induction.

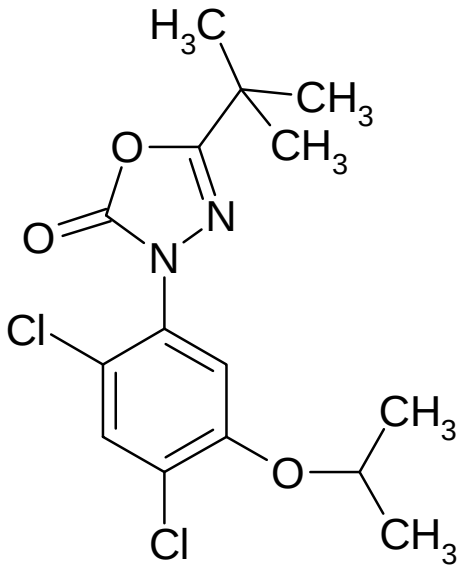
B. Structural Activity Relationships

The two structurally related compounds, azafenidin and sulfentrazone, are nonmutagenic and are not hepatocarcinogens in rats or mice.

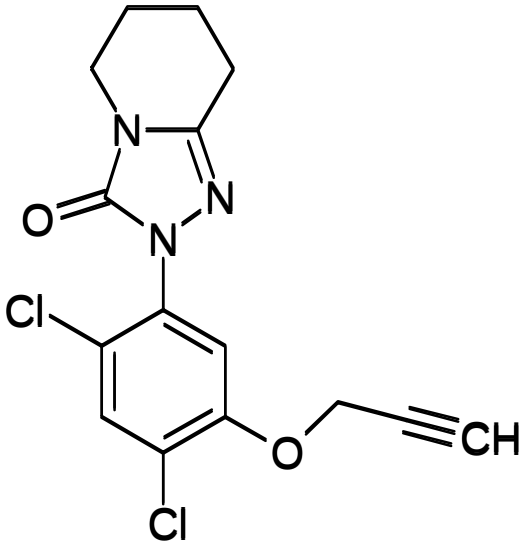
Oxadiazon belongs to the class of oxadiazoles. While oxadiazon is only somewhat similar in structure to other pesticides that have a chlorinated phenol ring and a heterocyclic ring (Figure 2), it shares some biological properties with azafenidin and sulfentrazone (i.e., inhibition of protoporphyrinogen oxidase and/or adverse effects on the hematopoietic system and the liver of rodents). Sulfentrazone produced equivocal results in the mouse lymphoma forward mutation assay, was not mutagenic in *Salmonella* and was neither clastogenic nor aneugenic *in vivo*. There was also no evidence of a carcinogenic effect in 2-year bioassays in rats and mice (EPA, 1999). Azafenidin was not mutagenic in the available studies. The CARC agreed that the consensus classification for male rat thyroid tumors seen following administration of azafenidin should be in **"Data are inadequate"** category but no additional cancer studies were considered necessary nor would there be any quantification of human cancer risk.(CARC, 1999; HED Doc # 013794).

Figure 2. Structural Activity Relationships: Oxadiazon and Related Structures

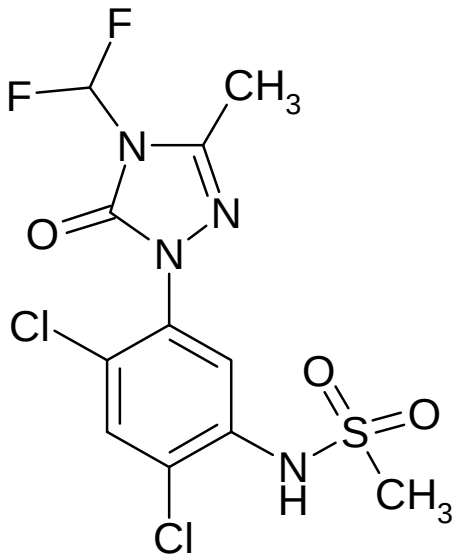
Oxadiazon



Azafenidin



Sulfentrazone



C. Mode of Action Studies

The Mechanism of Toxicity Assessment Review Committee (MTARC) determined that the available information does not support the proposed non-genotoxic mode of action for oxadiazon-induced liver tumors in rodents (McCarroll, 2001).

The mechanistic studies including an unpublished study in Sprague Dawley rats (MRID No. 42310001) and a published study by Richert *et al.*, 1996 in Sprague Dawley rats, CD-1 mice and Beagle dogs were presented to the MTARC on February 8, 2001. The committee concluded that peroxisome proliferation may be a possible mode of action for oxadiazon-induced liver tumors in rats and mice. However, there are deficiencies in the database which include lack of cell proliferation data in rat and mouse studies, lack of concordance between the dose response for peroxisomal enzymatic activity and liver tumor induction, and decrease rather than increase in catalase activity. Therefore, the available information is inadequate to determine the mode of action for oxadiazon-induced liver tumors in rodents.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity

The CARC concluded that:

- **In the combined chronic toxicity/carcinogenicity study in Wistar rats, there was clear evidence that oxadiazon was carcinogenic to male rats** because: 1) There were statistically significant ($p<0.01$) positive trends for liver adenomas, carcinomas and combined adenomas/adenocarcinomas. There was also a statistically significant ($p< 0.05$ or $p<0.01$) increase by pair-wise comparisons of the 100 and 1000 ppm (3.5 and 39 mg/kg/day, respectively) dose groups with the controls for liver adenomas (9% and 13% , respectively, vs 0% in controls) and combined adenomas/carcinomas (9% and 23% vs 0% in controls) as well as liver carcinomas (10% vs 0% in controls) at 1000 ppm; and 2) The incidences of liver adenomas and carcinomas were outside the range for the published spontaneous rates (range both for liver adenomas and carcinomas : 0%-2.5%). For females, there was a statistically significant increasing trend for liver carcinomas but there were no significant pair-wise comparisons of liver tumors in treated females.

  The highest dose level tested was considered to be adequate and not excessive for males based on decreased body weight gains as well as clinical and the clinical and histopatholgical liver changes observed which were not severely adverse. For female rats, the highest dose was considered to be adequate and not excessive based on an increased incidence of chronic nephropathy and liver changes. The survival of the animals was not affected by the treatment. **The CARC concluded that the increases in both the benign and malignant liver tumors in the male rats were treatment-related.**

- **In the combined chronic toxicity/carcinogenicity study in ICR-JCL mice, there was clear evidence that oxadiazon was carcinogenic to male and female mice** because: 1) There were statistically significant ($p<0.01$) positive trends for liver adenomas, carcinomas and combined adenomas/carcinomas in males and females. For males, there were also statistically significant ($p<0.05$ or $p<0.01$) increases by pair-wise comparisons of the 100 and 1000 ppm (10.6 and 113 mg/kg/day, respectively) dose groups with the controls for liver adenomas (17% and 23%, respectively, vs 3% in controls), carcinomas (33% and 63%, respectively, vs 4% in controls) and combined adenomas/carcinomas (16% and 41% , respectively, vs 7% in controls). For females, there were also statistically significant ($p<0.05$ or <0.01) increases by pair-wise comparisons of the 1000 ppm dose group (99 mg/kg/day) with the controls for liver adenomas (16% vs 0% in controls), carcinomas (14% vs 2% in controls) and combined adenomas/ carcinomas (29% vs 2% in controls). The increase in the incidence of lymphomas in females was not considered to be treatment-related because there was no dose-response, the tumor response was variable and this type of tumor was not seen in male mice or in earlier studies.

  The highest dose level tested for the male and female mice was considered to be adequate and not excessive based on the findings of anemia and histopatholgical

changes in the liver which were not severely adverse. There were no adverse effects on the body weight gain and survival of the animals. **The Committee concluded that the increases in both benign and malignant liver tumors in male and female mice were treatment-related.**

The positive results in an *in vitro* cell transformation assay are in concordance with the findings of liver tumor induction in the *in vivo* rodent studies.

2. Mutagenicity

The CARC concluded that oxadiazon was negative for mutagenic potential in a battery of acceptable mutagenicity studies which satisfy the pre-1991 FIFRA guideline requirements. These studies included reverse gene mutation assays in bacteria, a mouse lymphoma forward gene mutation assay, chromosome aberration assays, and UDS assays. No new studies were requested by the CARC.

3. Structure-Activity Relationships

The structurally-related compounds, azafenidin and sulfentrazone, are neither mutagenic nor liver carcinogens in rats or mice.

4. Mode of Action Studies

The MTARC concluded that the available mechanistic data are insufficient to determine whether oxadiazon-induced liver tumors in the rats and mice were associated with peroxisome proliferation. Nevertheless, the formation of brown pigment in the liver and kidneys of rats and mice is consistent with the inhibition of porphyrin biosynthesis by oxadiazon. It is caused by the disruption of the biosynthetic pathways producing heme that leads to accumulation of precursors throughout the body.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified oxadiazon into the category **"Likely to be carcinogenic to humans"** based on the following weight-of-the-evidence considerations:

1. Treatment-related benign and malignant liver tumors were observed in two species. There was clear evidence that oxadiazon induced both benign and malignant liver tumors in male Wistar rats and male and female ICR-JCL mice. Liver tumors were also noted in earlier studies with F-344 rats and CD-1 mice using higher doses of oxadiazon that exceeded the MTD.

2. Oxadiazon was not mutagenic. However, it causes cell transformation *in vitro;* these results are in concordance with the carcinogenicity seen in *in vivo* rodent studies.

3. The available mechanistic studies do not support a non-genotoxic mode of action for oxadiazon -induced liver tumors in rodents.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

.

For human cancer risk assessment, the CARC recommended using a linear low dose extrapolation approach based on the most potent liver tumors in the rats and mice. This approach is supported by the inadequacy of data on the mode of action for oxadiazon-induced liver tumors in rodents.

VII. BIBLIOGRAPHY

MRID No.	CITATION
40993401	Shirasu, Y. (1987). Oxadiazon - 24 Month Chronic Toxicity and Carcinogenicity Study in Rats. Institute of Environmental Toxicology, Tokyo, Japan; Study No. Not listed; Report dated February 1987. (Unpublished). HED Doc.# 08949.
40993301	Shirasu, Y. (1987). Oxadiazon-23 Month Chronic Toxicity and Carcinogenicity Study in Mice. Institute of Environmental Toxicology, Tokyo, Japan; Study No. Not listed; Report dated February 1987. (Unpublished). HED Doc.# 08949.
---	Brunsman, L.L. (2001). Oxadiazon Qualitative Risk Assessment (Q_1^*) Based on SPF Wistar Rat and ICR_JCL Mouse Dietary Studies. A memorandum from Lori L. Brunsman, Science Analyses Branch, Health Effects Division to Nancy McCarroll, Toxicology Branch, Health Effects Division, dated February 14, 2001. HED Doc # 014470.
---	CARC. (1999). Evaluation of the carcinogenic potential of Azafenidin/ Cancer Assessment Document. Cancer Assessment Review Committee , Health Effects Division, Office of Pesticide Programs. Final Report dated October 18, 1999. HED Doc # 013794.
---	EPA (1999). Office of Pesticide Programs List of Chemicals Evaluated for Carcinogenic Potential. Memorandum from William Burnam to Division Directors, Office of Pesticide Programs, Environmental Protection Agency. August 25, 1999.
---	Farber, T.M.. (1987). Classification of Oncogenic Potential of Oxadiazon. Memorandum from Theodore M. Farber, Health Evaluation Division to Edwin F. Tinsworth, Registration Division, Office of Pesticide Programs, U.S. EPA., dated August 27, 1987. HED Doc # 007798.
---	Litt, B.D. (1984). Carcinogenicity Risk Assessment for Oxadiazon. Memorandum from Bertram D. Litt, Toxicology Branch to Richard Mountfort Registration Division, Office of Pesticide Programs, U.S. EPA., dated November 21, 1984. HED Doc# 004097.
---	Matringe, M., Camadro, J.M., Labbe, P., Scalla, R. (1989). Protoporphyrinogen oxidase inhibition by three peroxidizing herbicides: oxadiazon, LS 82-556 and M&B 39279. FEBS Letters 245, number 1, 2:35-38.

---------- McCarroll, N. (2001). Oxadiazon: Assessment of Mode of Action on Liver Carcinogenicity. A memorandum from Nancy McCarroll, Toxicology Branch to William Burnham, Immediate Office, Office of Pesticide Programs, U.S. EPA., dated February 28, 2001.

---------- Quest, J.A. (1987). 2nd Peer Review of Oxadiazon. A memorandum from John A. Quest, Toxicology Branch to Richard Mountfort Registration Division, Office of Pesticide Programs, U.S. EPA., dated April 14, 1987. HED Doc # 07798

-------- Richert, L., Price, S., Chesne, C., Maita, K. Carmichael, N. (1996). Comparison of the induction of hepatic peroxisome proliferation by the herbicide Oxadiazon *in vivo* in rats, mice, and dogs and *in vitro* in rat and human hepatocytes. Toxicol. Appl. Pharmacol 141: 35-43.

-------- Welsh, K. M. And Poteracki, J. (1994). Spontaneous neoplasms in control Wistar rats. Fundam Appl Toxicol 22:65-72.